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Comparative aspects of the transport of immunoglobulin A from blood to bile

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Summary. Polymeric and monomeric human IgA were isolated from the sera of patients with IgA myeloma; rat IgA polymer, monomer and IgG2 were isolated from the ascitic fluid or sera of Lou/Wsl rats bearing appropriate myelomata. The purified Ig preparations were labelled with ^{125}I and injected intravenously into rats, rabbits, guinea-pigs or sheep that had had a cannula inserted into the common bile duct so that their bile could be collected quantitatively. Rats and rabbits transported 30% of the injected dose of both IgA polymers, but no other type of immunoglobulin, from blood to bile within 5–7 h. Sheep and guinea-pigs were unable to transport any of the immunoglobulin preparations from blood to bile, even though the injected material remained circulating in the blood.

INTRODUCTION

The binding of polymeric IgA to secretory component (SC) on the deep surface of the enterocytes is followed by the internalization of the IgA–SC complex so that it is carried across the cytoplasm, probably inside vesicles, and discharged into the lumen of the gut (Brown, 1978; Nagura, Nakane & Brown, 1979). In rats, IgA also reaches the gut via the bile because it is rapidly and actively transported from the blood (Orlans, Peppard, Reynolds & Hall, 1978; Jackson, Lemaître-Coelho, Vaerman, Bazin & Beckers, 1978) by the hepatocytes (Birbeck, Cartwright, Hall, Orlans & Peppard, 1979). Like enterocytes, the hepatocytes have SC on their surfaces and apparently transport IgA in much the same way (Orlans, Peppard, Fry, Hinton & Mullock, 1979; Socken, Jeejeebhoy, Bazin & Underdown, 1979; Mullock, Hinton, Dobrota, Peppard & Orlans, 1979).

Since it is relatively easy to study the extent and kinetics of IgA transport by collecting bile, and since there is evidence that IgA and SC from different species will interact *in vitro* (Mach, 1970; Socken & Underdown, 1978) we have used this method to study the transport of heterologous IgA in four mammalian species.

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MATERIALS AND METHODS

General plan

Recipient animals were prepared with a cannula in the common bile duct so that the bile could be collected quantitatively. Another cannula was inserted into a vein so that an accurately measured and timed dose of ^{125}I-labelled immunoglobulin (Ig) could be injected easily and later samples of blood obtained. Rats and guinea-pigs received Ig doses of approximately 1 mg, rabbits 3 mg, and sheep 10 mg. After the injection the bile was collected at intervals of 30 min for at least 5 h and, where possible, blood was collected every hour. Blood from rats and guinea-pigs was taken into tared tubes, but volumetrically measured amounts of

plasma were collected from heparinized blood of rabbits and sheep. Before being assayed for radioactivity, the blood and bile samples were treated with 10% trichloracetic acid and the γ-emission of the precipitated protein was measured in a Wilj 2001 gamma counter (Wilj Electronics, Ashford, Kent).

Immunoglobulins

IgA polymer and IgG2 were isolated from the sera and ascitic fluid of Lou/Wsl rats bearing appropriate myelomata as described previously (Orlans *et al.*, 1978, 1979). Rat IgA monomer was isolated from the same material (Peppard, 1979). Samples of purified proteins were labelled with ^{125}I using chloramine T as described previously (Hall, Orlans, Reynolds, Dean, Peppard, Gyure & Hobbs, 1979) to an activity of 0·02 μCi/μg. Similarly prepared and radiolabelled human IgA dimer and monomer, from the serum of a patient with an IgA myeloma, were kindly supplied by Dr Eva Orlans and Jane Peppard.

Animals and surgical procedures

Male, Wistar rats weighing 200 g were taken from our own animal house as required. The methods of surgery, anaesthesia and restraint in Bollman cages have been described (Hall *et al.*, 1979). Out-bred, male Dunkin-Hartley guinea-pigs weighing 350-450 g were treated similarly.

Male New Zealand White rabbits weighing about 3·5 kg were anaesthetized with 0·3 ml/kg i.v. Hypnorm (Jansen Pharmaceuticals, c/o Crown Imperial Co., Lamberhurst, Kent). The abdomen was opened by a right subcostal incision, the cystic duct was tied off and a polyvinyl cannula was inserted into the common bile duct. After closing the abdomen the rabbit was placed in an ordinary bleeding-box for the duration of the experiment.

Yearling sheep, either wethers or ewes, were purchased at local auctions and penned in a purpose-built sheep house. Abdominal and lymphatic surgery was carried out as described previously (Hall, Hopkins & Orlans, 1977; Hecker, 1974). Sheep with cannulated common bile ducts were kept in individual metabolism cages and were not used for experiments until at least 24 h after the operation.

RESULTS

Technically successful experiments were carried out on twelve rats, six guinea-pigs, five rabbits and five sheep.

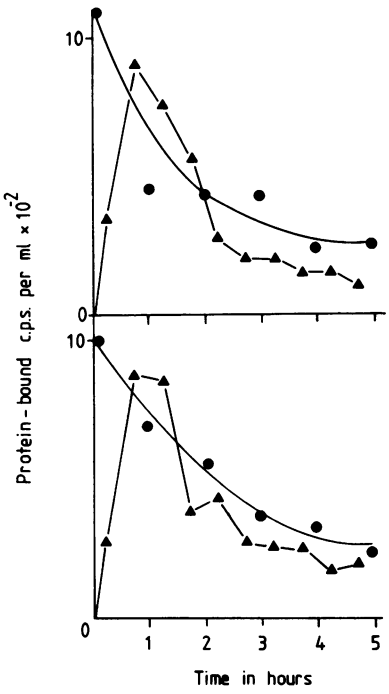

Figure 1. The protein-bound radioactivity (counts per second per ml) of whole blood (●) and bile (▲) of rats after ^{125}I polymeric IgA had been injected intravenously at time zero. The upper graph shows the results from an experiment where homologous (rat) IgA had been injected; the lower graph shows the results of injecting heterologous (human) IgA. In both experiments, just over 30% of the injected radioactivity was recovered in the bile.

The transport of homologous, polymeric myeloma ^{125}I-IgA from blood to bile in the rat was used as the baseline with which other results were compared. When this material was injected intravenously it was cleared from the blood very rapidly; it started to appear in the bile within 20 min, and by 5 h over 30% of the injected dose had been recovered in the bile. This is illustrated in Fig. 1; also shown is the transport of dimeric human ^{125}I-IgA from blood to bile in the rat, the behaviour of the human IgA was similar to that of the homologous protein. Conversely, monomeric IgA from either species never appeared in the bile even though high levels of activity remained in the blood throughout the experiments. As in previous experiments (Orlans *et al.*, 1978), preparations of rat IgG did not appear in the bile either.

When either rat or human polymeric ^{125}I-IgA were injected i.v. into rabbits more than 30% of the injected doses appeared in the bile with similar kinetics as in the

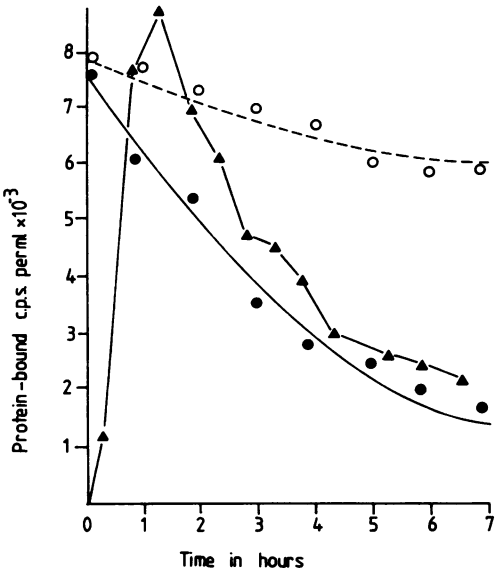

Figure 2. The protein-bound radioactivity (counts per second per ml) of the blood plasma (●) and bile (▲) of a rabbit after ^{125}I polymeric rat IgA had been injected intravenously at time zero. During the 7 h or the experiment, 35% of the injected dose of IgA was recovered in the bile. An identical set of results was obtained when ^{125}I polymeric human IgA was injected. The broken line (o- - - -o) shows the radioactivity in the blood of a rabbit after ^{125}I rat IgG2 had been injected; none of this material appeared in the recipient's bile.

rat (Fig. 2). Again, in rabbits as in rats, preparations of IgA monomer or IgG were not transported into the bile.

In contrast to the situation in rats and rabbits, neither guinea-pigs nor sheep transmitted any class of human or rat Ig from blood to bile. The injected doses of IgA polymers remained in the blood and, in sheep with lymphatic fistulae, were shown to equilibrate between blood and lymph in a perfectly normal fashion. Their failure to appear in the bile thus cannot be attributed to their failure to circulate and diffuse normally.

DISCUSSION

The total failure of sheep and guinea-pigs to transmit heterologous IgA polymers from blood to bile contrasts with the ability of rats and rabbits to do so. The active transport of human myeloma IgA by rat liver has been described (Vaerman & Lemaître-Coelho, 1979) and the present results show that rabbits too can transmit heterologous IgA from blood to bile, and that in both species it is transmitted at the same rate as is homologous IgA in the rat.

Brandtzaeg's (1974) proposal that SC is properly regarded as a receptor for IgA on the membrane of enterocytes has now been extended to hepatocytes (Orlans *et al.*, 1979; Socken *et al.*,1979). Accordingly, our results could be explained by the assumption that, under *in vivo* conditions, the SC on the hepatocytes of rats and rabbits cannot distinguish between native and human (and/or rat) IgA. Indeed, interactions *in vitro* between rabbit SC and human IgA jave been described (Socken & Underdown, 1978) but, unfortunately for any simple unifying theory, these authors also found some interaction between sheep SC and human IgA, and similar results were reported by Mach (1970). Our *in vivo* results argue against a true functional interaction between sheep SC and rat or human IgA but, in any case, the situation in sheep is complicated. We have been unable to detect much intact immunoglobulin of any class in sheep bile and there is thus some doubt that sheep transport even their own IgA from blood to bile on a really substantial scale.

We have found that although guinea-pig bile is relatively dilute in respect of protein, it flows rapidly so that the amount of protein excreted per unit time in the bile is comparable to that in the rat. Polyacrylamide gel electrophoresis indicated that much of the globulin in guinea-pig bile could be accounted for by a protein of the same size as other mammalian IgAs. It seems likely, therefore, that guinea-pigs transport their own IgA from blood to bile even though they cannot transport human or rat IgA.

Thus, although the observed results were unequivocal they are not susceptible to a simple explanation, and emphasize the dangers of generalizing about mechanisms of secretory immunity in mammals.

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